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Docking, Microwave Assisted Synthesis of Benzothiazole Derivatives using Green Solvent as Antioxidant Agent

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Abstract: Several green chemistry approaches are used in drug and chemical synthesis, including microwave-assisted solvent and catalyst-free processes. These procedures are pure, cost-effective, friendly to the environment, and provide great yields. The current work focuses on using green chemistry approaches for the production of benzothiazole derivatives. Benzothiazole derivatives were synthesised using microwaves as a green chemistry strategy and glycerol as green solvent. The reaction was verified by TLC and characterised using FTIR spectra. The antioxidant activity of benzothiazole derivatives was determined by using DPPH. Compound BT3 and BT5 showed excellent antioxidant potential. Molecular docking was done for derivatives on human peroxiredoxin 5 (PDB ID: 1HD2) and produced an excellent docking score.

Keywords: Green chemistry, Microwave aided synthesis, Benzothiazole, Green solvent, Antioxidant activity, Molecular docking

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Introduction

Green chemistry is a recent branch of chemistry that unites tools and methods to support chemical engineers' research into the development of chemical products and processes that lessen or do away with the use of hazardous chemicals (Patil *et al*., 2017) This enables them to create more efficient and effective products with less waste (Warner *et al*., 2004) Green chemistry is now crucial to the field of synthetic chemistry (Shrivastava, 2012; Hazra, 2021)

Benzothiazole is a sulfur containing hetero-cycle which contains a benzene ring which is fused

to a thiazole heterocyclic ring. The benzothiazole ring structure, which is usually employed as synthetic rubber, was initially identified in different marine and terrestrial natural substances (Gao *et al*., 2020). It provides a wide range of biological functions in the area of medicinal chemistry, including anti-tuberculosis, anti-diabetic, anti-cancer, anthelmintic, anti-bacterial, anti-oxidant, anti-tuberculosis, anti-viral, anti-inflammatory, anti-glutamate, anti-tumor and neuroprotective, anticonvulsant, muscle relaxant, anti-parkinsonism. It also act as enzyme inhibitors, anti-inflammatory drugs, plant growth regulators and antioxidants (Ali and Siddiqui, 2013; Djuidje, 2022). The current work focuses on using green chemistry approaches for the production of benzothiazole derivatives.

Materials and Methods

All reagents and solvents were used without further purification. All the recorded melting points were taken in an open glass capillary on a Griffin apparatus, and the values given were uncorrected. The structure of the synthesized derivatives was determined using spectral analysis like IR.

IR spectra were determined using potassium bromide discs, and values were represented in cm^{-1}. IR spectra were recorded on Shimadzu IR 435 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The reaction progress was monitored by TLC using precoated silica gel aluminium sheets (Merck). The spots were visualized using a UV Lamp. The solvent system used for TLC was n-hexane and ethyl acetate, with a ratio of 9:1.

Synthesis of 2-Substituted Benzothiazole:

A mixture of 2-aminophenol (4 mmol), substituted aromatic aldehyde (4 mmol), glycerol (4 ml) was placed in a microwave flask and irradiated at 170 watts. TLC was used to confirm that the reaction was complete. After the reaction was complete, the mixture was cooled, and then mixture of water and ethylacetate were added onto mixture. The crude product was separated and recrystallized (Zhang *et al*., 2012).

Antioxidant activity:

The antioxidant activity of all synthesised compounds was determined using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. (de Torre *et al*., 2019). DPPH solution was prepared by dissolving 2 mg of DPPH in 100 ml of methanol. Concentrations of ligands and ascorbic acid (positive control) was ranging from 25 to 100 g/ml. To investigate antioxidant activity, 2 ml of DPPH and 2 ml of each ligand were mixed and incubated in the dark. The radical scavenging capacity was measured with a UV-visible spectrophotometer after 15, 30, and 45 min. The activity was determined by the fraction of scavenging activity (Mali *et al*., 2022) obtained from the following equation:

$$\% \text{ Scavenging activity } = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

Docking study:

Based on a literature review, oxidoreductase protein molecules were selected as the target molecule for the docking investigation. The 3D structure of target proteins (1HD2) (Dincel *et al*., 2020) was retrieved from the Protein Data Bank (www.rcsb.org). The docking compatible structures of synthesised benzothiazole derivatives (ligands) were first sketched in ChemDraw (Patil and Amrutkar, 2021), which was then translated to OpenBabel 3.1.1. (O'Boyle *et al*., 2011). The active domain of a protein molecule was found using the Pymol programme. The target proteins were prepped for docking using the Autodock tool 1.5.7, which removed water, heteroatoms, and added charges (Allouche, 2012) The ligand structure was docked to the active domain, and post-docking analysis was carried out in the Discovery Studio 3.5 Visualizer (DSV) (Biovia, 2017).

Results and Discussion

Chemistry:

Microwave-assisted organic synthesis has significantly changed the way chemists approach organic synthesis. Glycerol is abundant, biodegradable, cheap, environmentally friendly, non-

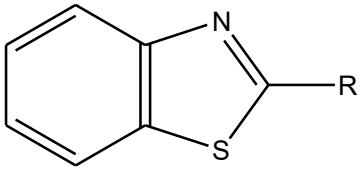
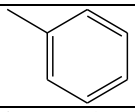
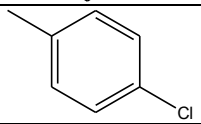
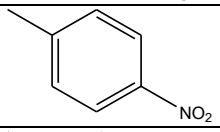
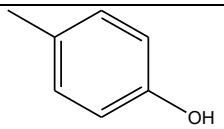
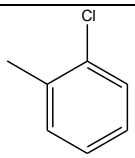


Fig. 1: General Structure of 2- substituted Benothiazole.

Table1: Physical characteristic of synthesised 2-substituted benzothiazole

Comp Code	IUPAC name	R	Molecular formula	Molecular weight	Crystal shape
BT1	2-Phenyl benzothiazole		$C_{13}H_9NS$	211.28	Needle shape
BT2	p-Chlorophenyl Benzothiazole		$C_{13}H_{18}ClNS$	245.73	Needle shape
BT3	p-Nitrophenyl Benzothiazole		$C_{13}H_8N_2O_2S$	256.28	Needle shape
BT4	p- Hydroxyphenyl Benzothiazole		$C_{13}H_9NOS$	227.28	Needle shape
BT5	o-Chlorophenyl Benzothiazole		$C_{13}H_8ClNS$	245.73	Needle shape

flammable, and has a high boiling point. It is extremely polar and immiscible with hydro-carbons and ethers.

It is ideal for biphasic catalysis and product separation by liquid-liquid extraction. A suitable green solvent Glycerol is used. In comparison to the traditional heating approach, benzothiazole synthesised in a microwave oven with glycerol as a green solvent resulted in a higher yield and improved quality.

Physical characteristic of synthesised 2-substituted benzothiazole:

Table 1 summerises the physical characteristic of synthesised 2-substituted benzothiazole and Fig. 1 shows the General Structure of 2- substituted Benothiazole.

2-Phenyl benzothiazole (*BT1*):

White needle shape shinny crystal, 85.66%, m.p.-110-114^{0}C, Rf-0.4, IR (KBr) cm^{-1}: 3062.89 (C–H, Ar), 1624.45(C=N str.), 1306.18(C-N str.), 740.45 (C-S str.)

p-Chlorophenyl Benzothiazole (*BT2*):

Buff colour needle shape crystal 77.55%, m.p.-118-120^{0}C, Rf-0.25, IR (KBr) cm^{-1}: 3061.93 (C–H, Ar), 1604.81(C=N str.), 1302.72(C-N str.), 742.47 (C-S str.), 829.58 (C-Cl str.)

p-Nitrophenyl Benzothiazole (*BT3*):

Yellow colour needle shape crystal 79.01%, m.p.-266-268^{0}C, Rf-0.3, IR (KBr) cm^{-1}: 2984.03 (C–H, Ar), 1625.80(C=N str.), 1290.45(C-N str.), 740.36

Table 2: Percentage of inhibition of DPPH by Benzothiazole derivative

Sample ID (Conc.)	Per cent inhibition		
	After 15 min.	After 30 min.	After 45 min.
Std (50)	29.50	32.36	42.26
Std (75)	31.10	32.89	45.26
Std (100)	34.56	33.65	48.26
BT1 (50)	10.11	12.26	15.26
BT1(75)	15.10	12.98	16.68
BT1(100)	14.13	15.26	18.26
BT2 (50)	22.58	27.59	30.25
BT2(75)	23.25	28.56	31.26
BT2(100)	25.46	28.96	33.59
BT3 (50)	30.05	31.56	45.26
BT3(75)	31.26	32.26	48.36
BT3(100)	31.65	33.82	48.97
BT4 (50)	19.26	20.89	33.69
BT4(75)	20.56	21.46	35.62
BT4(100)	20.98	22.05	36.59
BT5 (50)	31.25	38.26	55.26
BT5(75)	32.62	39.26	58.26
BT5(100)	33.26	40.00	59.06

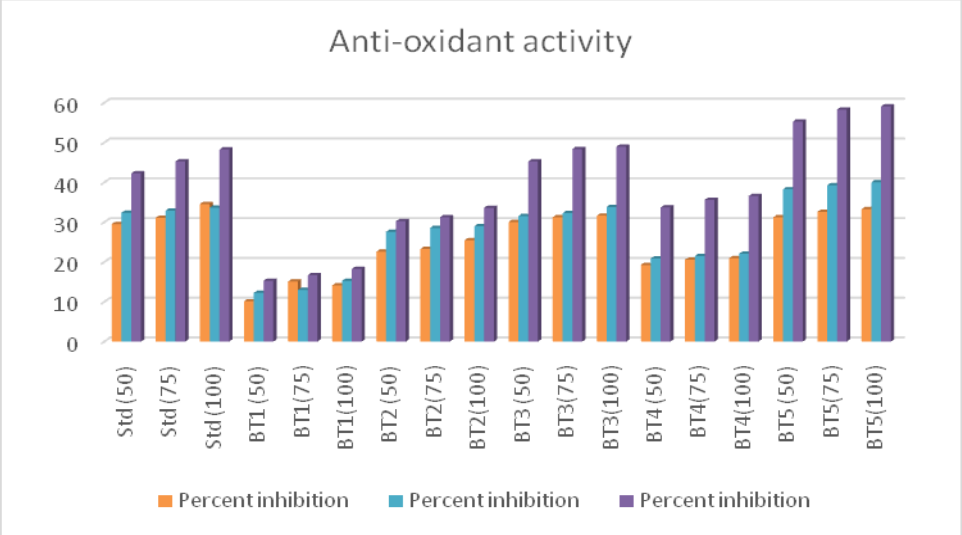


Fig. 2: Antioxidant activity of 2-amino thiazole derivatives by DPPH method.

(C-S str.), 1363.40 (N=O str.)

p- hydroxyphenyl benzothiazole (*BT4*):

White needle shape shinny crystal, 85.66%, m.p.-110-114^{0}C, Rf-0.4, IR (KBr) cm^{-1}: 3329.26 (O–H, str), 1631.84 (C=N str.), 1293.71(C-N str.), 744.55 (C-S str.), 1200.26 (C-O)

o-Chlorophenyl Benzothiazole (*BT5*):

Buff colour needle shape crystal 77.55%, m.p.-118-120^{0}C, Rf-0.25, IR (KBr) cm^{-1}: 3063.30 (C–H, Ar), 1699.06(C=N str.), 1306.40(C-N str.), 742.47 (C-S str.), 727.92 (C-Cl str.)

Antioxidant Activity:

The biological assessment of the synthesised derivative for antioxidant activity was performed using the well-known DPPH technique, with ascorbic acid as the reference. Antioxidant activity was determined by measuring UV absorbance,

Table 3: Binding Affinity and Interaction of Benzothiazole Derivatives with Target Protein

Compound Name	Binding Energy (Kcal/Mol)	Inhibition Constant (Ki)	Hydrogen Bonding	Amino Acid Involved In Hydrogen Bonding Interaction	Type of Interaction	Amino Acids
p-Hydroxyphenyl benzothiazole	6.21	27.89	3	LYS A:63ALA A:59VAL A:70 VAL A:69	Van Der Waals, Pi-Sigma, Pi-Alkyl, Pi-Cation Conventional H Bonding	GLN A:68 LYS A:63 ALA A:59 VAL A:70 VAL A:69 GLY A:92 LEU A:62 LYS A:93 ARG A:95 VAL A:67
P-Nitrophenyl benzothiazole	8.03	1.29	3	LYS A:63 VAL A:70GLN A:68ARG A:95 GLU A:16ALA A:90GLY A:92	Van Der Waals, Pi-Sigma,Pi-Alkyl,Pi-Anion, Conventional H Bonding, Unfavourable acceptor	LYS A:63 VAL A:70 GLN A:68 ARG A:95 GLU A:16 ALA A:90 GLY A:92 LEU A:96 GLU A:91

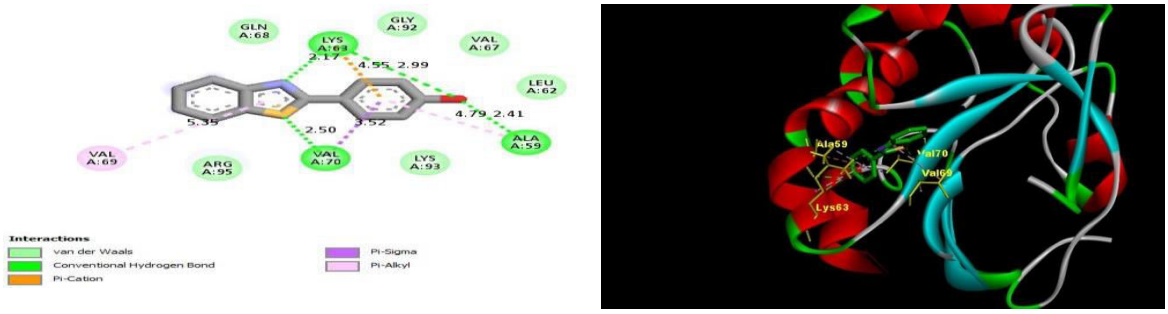


Fig. 3: 2d & 3d structure of p- hydroxyl phenyl benzothiazole and its active site of human peroxidise.

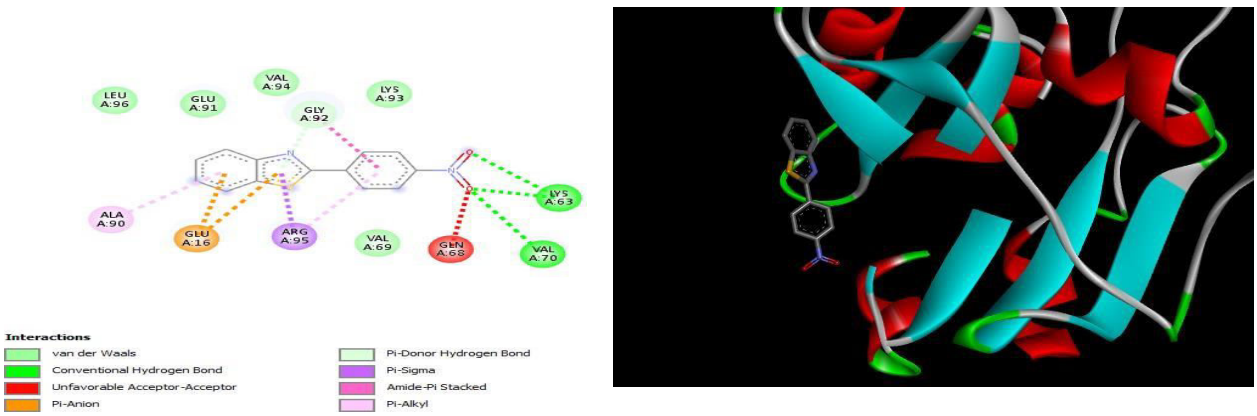


Fig. 4: 2d & 3d structures of nitrophenyl benzothiazole and PDB ID : 1HD2.

then calculating percent inhibition and comparing it to the reference medication, as indicated in Table 2 and Figure 2. All synthesised derivatives greatest antioxidant scavenging activity among the synthesised derivatives. The same confirmation is obtained by docking study.

Docking Study:

Peroxiredoxins are present in both prokaryotic and eukaryotic cells. Peroxiredoxin 5 (PRDX5) is very important redox enzyme present in tissues, including mitochondria, peroxisomes, and cytosol. PRDX5 has a role in both antioxidant protection and cell signalling. Hence, Human peroxiredoxin 5 (PDB ID: 1HD2) was selected for molecular docking.

Using the *in vitro* data, extensive docking experiments were performed to investigate the possible ways of binding of the highly active compounds BT3 and BT4 inside the active region of human peroxiredoxin 5 (PDB ID: 1HD2). We effectively recreated the experimental binding conformations of Benzothiazole in the binding pocket of 1HD2 within an acceptable range. Table 3 summarized the bonding interaction and bonding energy of synthesised compounds. Figure 2 shows how compound BT3 interacts with the active region of Human Peroxiredoxin 5 (PDB ID 1HD2). BT3 was surrounded by amino acid residues from the receptor's active site, including LYS 63, ALA59, VAL 69 and VAL 70. Also, the hydroxyl group is involve in stronger interaction like hydrogen bonding.

Conclusion

This approach is ideal for synthesising benzothiazole derivatives because of its high yields, quick reaction times, mild reaction conditions, and simple work-up processes. The study concluded that 2- substituted benothiazoles has potent antioxidant potential as evaluated by DPPH assay. IF it is substituted with electron withdrawing substituents like nitro, hydroxyl activity is enhanced. The findings of this work showed the true potential of the benzothiazole have a significant antioxidant capability when compared to conventional ascorbic acid. Compound BT3 has greater and BT5 had the scaffold when adequately substituted as a foundation for the construction of multifunctional molecules. Molecules BT3 and BT5, in particular, provided promising markers for the synthesis of more potent molecules through additional structural alterations.

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